THE EFFECTS OF LASER THERAPY ON INSULIN SECRETION IN ISOLATED PORCINE ISLETS

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Finally, I would like to share my sincere appreciation to all my supportive professors who have been a continuous source of encouragement during my research project, as well as my seven years as a student at Angelo State University.

ABSTRACT

Xenotransplantation of porcine islets to human recipients is being investigated as a potential cure for type 1 diabetes. The objective of the study was to evaluate effects of laser therapy on insulin secretion in pig islets. Sixteen pig pancreata were harvested for islet isolation. Islets were treated with laser dosages of 5J - 25J followed by 30, 20 or 10-minute incubation in low (3.0 mM) or high (16.7 mM) glucose. Insulin secretion was higher in samples exposed to 5J laser therapy followed by a 30-minute culture in low glucose ($P \leq 0.05$). When evaluating gender differences, male islets had an increased insulin secretion in low glucose ($P \leq 0.05$) compared to females. No significant differences were seen in high glucose. In conclusion, laser therapy increased insulin secretion on pig islets in low glucose media. Future research should further investigate effects of 5J and 10J laser dosages on male pig islets.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Type 1 Diabetes

Diabetes affects more than 300 million people across the world with an estimated 1.5 million people suffering from type 1 diabetes in the United States alone (Sherwin and Jastreboff, 2012; Van der Windt et al., 2012). Type 1 diabetes is a chronic condition where the pancreas produces little to no insulin. This is due to the body's immune system destroying the islets in the pancreas. This results in high concentrations of glucose in the bloodstream which can cause hyperglycemia. Hyperglycemia can cause adverse issues such as heart failure, stroke, blindness, kidney damage and nerve damage (Dokken, 2008; Pietrangelo and Cherney, 2017). The current treatment for type 1 diabetes is insulin injections or an insulin pump to help regulate glucose levels within the body.

In a healthy individual, the body breaks down carbohydrates into glucose which then gets passed into the bloodstream for use in the body's tissues as an energy source. The islets in the pancreas detect the rise in glucose present in the bloodstream and begin to secrete insulin from β cells within the islets. Insulin is the hormone that allows the body's tissue to absorb glucose.

Islet Transplantation

Pancreatic islet transplantation is a potential cure for type 1 diabetes. Compared to a pancreas transplantation, islet transplantation is not as invasive and is more easily tolerated (McCall and Shapiro, 2012). The expected outcome is insulin independence and stabilized

blood glucose resulting in transplantation as a promising treatment for future diabetes patients (Zinger and Leibowitz, 2017). However, there are a limited number of suitable donor pancreata, so the number of patients able to be treated is also small (Van der Windt et al., 2012). Another major obstacle faced includes the loss of healthy, functional islets during transplantation due to a high rejection rate of the porcine islets by the immune system of the recipient (Irani et al., 2009). Immunosuppression is required for the immune system to accept the transplanted islets; however, this increases the risk of infection for the recipient. Inflammation has also been seen after islet transplantation due to the body's natural inflammatory response. While human islet transplantation has proven to be successful in limited cases, research is needed to improve this success rate. Therefore, porcine (pig) islets are being considered due to their shared similarities with humans which include omnivorous habits as well as similar metabolic and cardiovascular features (Torres-Rovira et al., 2012). The use of pig islets for research holds great promise for largescale application of islet transplantation (Ferrer et al., 2008).

Xenotransplantation

Xenotransplantation is the process of transplanting organs or tissues between different species. It has been previously proven that transplanted pig islets could uphold normal glycemia levels in diabetic non-human primates for a period greater than 6 months and in some cases up to 1 or 2 years. A study utilizing an *in vitro* perifusion system found isolated pig islets demonstrate a biphasic insulin secretion pattern stimulated by glucose, which is a common pattern also found in insulin secretion of humans (Cooper et al., 2016). However, islet xenotransplantation faces many challenges. One of the main challenges includes the fact

that pig islets have poor sensitivity to glucose which results in poor insulin production when compared to human islets (Cooper et al., 2016).

Objective

Previous research found that phototherapy has increased the amount of insulin secreted when applied to islets. Therefore, the objective of this study was to evaluate the effects of laser therapy on isolated porcine islets in low and high glucose media.

LITERATURE REVIEW

Phototherapy

Phototherapy has been found to increase cellular proliferation of fibroblasts and stimulate secretion from secretory cells (Kamrani et al., 2008). These effects can be explained by the increase in adenosine triphosphate (ATP) which results in DNA synthesis. The content of ATP within the cell is a significant factor for increasing the islet insulin secretion (Kamrani et al., 2008). This is observed by the preserving characteristic phototherapy has on the mitochondria within the cell (Oron et al., 2007). Previous research has evaluated the effects of infrared light and laser therapy, specifically.

Infrared Light

Infrared (IR) light's impact on increasing insulin secretion in rats was found to not cause any damage to cell viability. Islets exposed to IR light did not have a decreased final insulin production normally observed after incubation (Meulenaer et al., 2009). On the second day after exposure, the number of cells recovered was significantly higher after exposure to IR because it promotes and favors growth of cells. Treatment with IR light had several favorable effects on the function of islets including the stimulation of cell growth, an increase in intracellular ATP content and insulin production (Meulenaer et al., 2009).

Low Level Laser Therapy

Low level laser therapy (LLLT) could improve rodent islet viability and function prior to transplantation (Irani et al., 2009). Many studies have shown *in vitro* laser therapy can accelerate the repair process as well as stimulate rapid cellular reproduction (Kamrani et al., 2008). Increased intracellular ATP results in DNA synthesis and helps explain the effects

of the rapid cellular reproduction after LLLT. Also, LLLT can increase the function of insulin secretion as well as its viability due to the increase in intracellular ATP and calcium influx (Kamrani et al., 2008). It was also found that the islet walls were not damaged after the laser therapy.

MATERIALS AND METHODS

Porcine Pancreas Procurement

A total of 16 pancreata from adult pigs were collected at a USDA inspected animal harvest facility. Animals were harvested under USDA humane handling and harvest protocols; as such IACUC approval requirements have been waived by the IACUC committee. After animal harvest, pancreas procurement began by cutting small masses of the pancreas out and trimming the mass into smaller pieces approximately 2.54 cm^3 in size. These pancreas sample pieces were placed into a CoStorSol University of Wisconsin (UW) cold storage washing solution (Preservation Solution Inc., WI, USA) for 5 seconds. The pieces were then placed into a sterile container containing 35 mL of fresh UW washing solution.

Islet Isolation and Culture

Islet isolation was completed in two distinct steps: pancreas digestion and islet purification. Pancreas digestion included eliminating other pancreatic cells from the sample by sectioning the pancreas and then exposing it to enzymatic digestion. This process began by trimming the pancreas samples to eliminate adipose and connective tissue approximately 40 to 60 minutes after harvest. Once all extra tissue was trimmed off, the pancreas was cut into smaller pieces and transferred to new sterile tubes where 15 mL UW solution was added. The islet purification step involved procuring the remaining islets to ensure higher quality samples and results for testing (McCall and Shapiro, 2012). This was done by adding a concentrated enzyme solution, Liberase DL (Sigma Aldrich, USA), in the concentration of

0.83mg/mL to promote digestion of the pancreas tissue. These tubes were incubated on a rotator at 37°C for 30 minutes.

Enzymatic digestion was stopped by adding cold Roswell Park Memorial Institute (RPMI) medium including 5% newborn calf serum (NCS) to the full volume of the tube. The RPMI medium is commonly used in cell cultures and tissue cultures because the medium is designed to promote the growth of mammalian cells. The solution was mixed slowly for 30 seconds and then stood still for 3 minutes to allow the tissue samples to settle completely to the bottom. The supernatant (SN) was pipetted out into a waste beaker. This washing process occurred one more time. Fresh RPMI medium with 5% NCS was added to reach a total volume of 30 mL and the tubes were placed in a centrifuge at 1500 G for 3 minutes at 4°C.

The SN was poured off into a waste beaker and plain UW solution was added to the tissue sample for a total volume of 12.5 mL. A working optiprep solution (1 part optiprep with 1 part plain UW solution) was added in the amount of 10 mL to each sample followed by adding 8 mL of an optiprep RPMI-1640 medium solution (9.1 mL optiprep, 25 mL plain RPMI and 0.696 mL of HEPES (a zwitterionic organic chemical buffering agent)). Each tube was filled to reach a total volume of 40 mL using plain RPMI medium solution and centrifuged at 500 G for 5 minutes at 4°C. The islets were transferred to 150 mm dishes containing 20 mL of an RPMI medium solution that includes 10% porcine serum and 1% of Anti-Anti (antibiotic-antimycotic) solution. Samples were cultured overnight in a 37°C and 5% CO_2 environment. The number of islets collected from each pancreas varied; therefore, this impacted the number of islet samples able to be tested within each pig.

Low Level Laser Therapy on Islets

Study 1: The islets were transferred back into tubes (2 dishes per tube) and placed in the centrifuge at 4500 G for 3 minutes at room temperature (RT). The SN was removed until approximately 1 to 2 mL was left in the 50 mL tube and each tube was transferred into a microcentrifuge tube. The microcentrifuge tubes were spun down at a benchtop centrifuge (Labnet International Inc., Cat. No.: C1201) at 6000 rpm for 30 seconds at RT. The SN was removed completely and 600 µL of plain RPMI medium solution was added to the islet samples. Next, 75 µL of the islet sample from each tube was transferred into eight separate wells in a 96-well microplate. A low glucose (3.0 mM) and high glucose (16.7 mM) media was made by using the RPMI medium solution (Appendix). The glucose media was added to the samples in designated wells. The laser (JDSU HeNe, model number: 1508-1) treatment was applied at various treatment time intervals, and the islets were cultured for 30 minutes in a 37°C environment with no cover (Table 1).

Study 2: The isolation process from Study 1 remained the same. Differences were seen when the islet samples were divided roughly into thirds before being loaded to the microplates. Each third of the islet sample was placed into 6 separate wells in one of the 96-well microplates. The same low and high glucose media from study 1 was made and added to the samples in designated wells. The laser treatment was applied at various treatment time intervals, and the first microplate was cultured for 30 minutes in a 37°C environment with no cover. The second and third microplates also received the same laser treatment at various treatment time intervals. The second microplate was cultured for 20 minutes and the third microplate was cultured for 10 minutes, both in a 37°C environment with no cover (Table 2).

Table 1. Levels of Laser Therapy Applied to Isolated Porcine Islets in Low and High Glucose Concentrations Based on Time Exposed to Laser for Study 1

Laser Dosage: 633 nm (joules)	Low Glucose: 3.0 mM (seconds)	High Glucose: 16.7 mM (seconds)
0	0	0
5	9	9
10	18	18
15	27	27
20	36	36
25	45	45

Table 2. Levels of Laser Therapy Applied to Isolated Porcine Islets in Low and High Glucose Concentrations Based on Time Exposed to Laser for Study 2

Laser Dosage: 633 nm (joules)	Low Glucose: 3.0 mM (seconds)	High Glucose: 16.7 mM (seconds)
0	0	0
5	9	9
10	18	18

After the culture period, the samples were then collected into microcentrifuge tubes and placed in the benchtop centrifuge at 6000 rpm for 60 seconds at RT. The SN was collected for an Insulin ELISA assay and placed into a separate tube leaving behind the islets in the original tube. All samples underwent snap freezing using liquid N_2 and placed in a -80°C freezer for future testing.

Insulin Secretion Assay

A Porcine/Canine Insulin ELISA assay (ALPCO, NH, USA) was utilized to measure the concentration of insulin present in samples from both study 1 and 2. The kit contained a conjugate stock, wash buffer concentrates and two controls (levels 2 and 3) that were prepared for future use. Each standard, control and SN samples were pipetted into their respective wells on a 96-well microplate at a volume of 25 µL each. A working strength conjugate stock was added to each well at a volume of 75 µL. The microplate was covered with a microplate sealer and incubated at RT for 2 hours, shaking at 700-900 rpm on a microplate shaker. At the end of the 2 hours, the contents of the wells were decanted, and the microplate was washed 6 times with the working strength wash buffer. A TMB substrate solution was added to each well at a volume of 100 µL, the microplate was covered again and incubated for 15 minutes at RT, shaking at 700-900 rpm on a microplate shaker. Once this is done, 100 µL of a Stop solution was added to each well of the microplate and the absorbance was measured using a microplate reader (BIO RAD iMark®, VT, USA).

Total Protein Extraction

Insulin secretion was normalized by measuring the total tissue volume (total protein concentration) to account for variation in the number of islets for each sample. This was

completed by setting the islet samples on ice while a Triton extraction buffer (100 µL of 10 mM TRIS HCL, 300 µL of 150 mM NaCl, 100 µL of 5mM EDTA, 200 µL of 20 mM Na Molybdate, 1000 µL of 50 mM Na Fluoride, 20 µL of 0.2mM Na Orthovanidate, 1000 µL of 2% Triton X-100, 7280 µL of Di H_2O and 10 µL of phenylmethylsulfonyl fluoride (PMSF)) was made. Once made, 100 µL of the extraction buffer was added to each islet sample and mixed thoroughly to initiate the breakdown of the cell membrane and to expose the protein concentration. The samples were then placed back on ice for 30 minutes before being centrifuged at 16,000 G at 4°C for 30 minutes.

After centrifugation, the SN was extracted out and into a new tube leaving behind the remaining broken-down cell components. The SN was run through a BCA Protein Assay Kit (Pierce, IL, USA) to determine the protein concentration present. The standards and samples were pipetted into their respective wells on a 96-well microplate at a volume of 25 µL each. A working reagent (WR) was prepared using two reagents (reagent A and B) provided in the assay kit into a concentration of 50 parts reagent A to 1 part reagent B. Two hundred µL of the WR was added into each well and the entire microplate was placed on a microplate shaker for 30 minutes. The microplate was then covered and incubated at 37°C for 30 minutes. At the end of the 30 minutes, the microplate was cooled to RT and the absorbance was measured using a microplate reader (BIO RAD iMark®, VT, USA). Variation in insulin secretion/total protein concentrations between each pig was observed, therefore each pig was normalized to its own control before comparing to other pigs.

Statistical Analysis

Data was analyzed using IBM-SPSS Statistics (version 21). For data sets with one

independent variable, a one-way ANOVA was initially applied. The data did not fit a normal distribution nor had equal variance. Therefore, Kruskal-Wallis, non-parametric test was applied. For data sets with two independent variables, a two-way ANOVA was initially applied. For those with an interaction, a one-way ANOVA with a Fisher's LSD post hoc test was applied where normal distribution and equal variance was met. Significant differences were determined by a P value of ≤ 0.05.

RESULTS

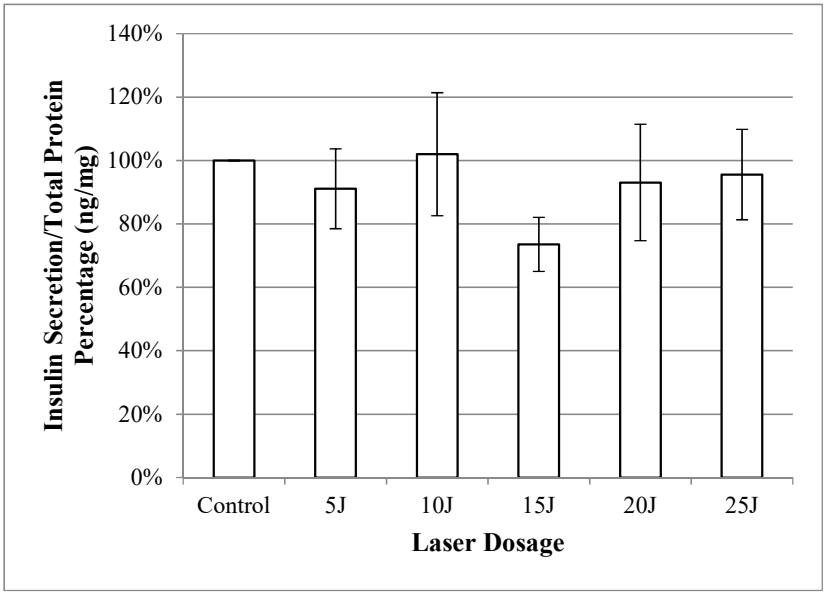
One Independent Variable

Multiple data sets were comprised based on comparisons of interest. First, data from Study 1 (n = 8) was ran through statistical analyses and no significant differences between laser treatment applications were found in either the low (Figure 1a) or high glucose media (Figure 1b). Three data sets from Study 2 (n = 8) were comprised from the three different incubation periods. The 30-minute incubation period showed significant differences (P = 0.043) in the low glucose; the 5J laser dosage increased the amount of insulin secreted compared to the control (Figure 2a). However, no significances were seen in the high glucose (Figure 2b). There were no significant differences seen in the 20-minute low (Figure 3a) and high glucose media (Figure 3b) or for the 10-minute low (Figure 4a) and high glucose media (Figure 4b). Data sets from combining 0J, 5J and 10J dosages from Study 1 and the 30-minute incubation period from Study 2 (n = 16) resulted in no significant differences in the low (Figure 5a) and high glucose media (Figure 5b).

Two Independent Variables

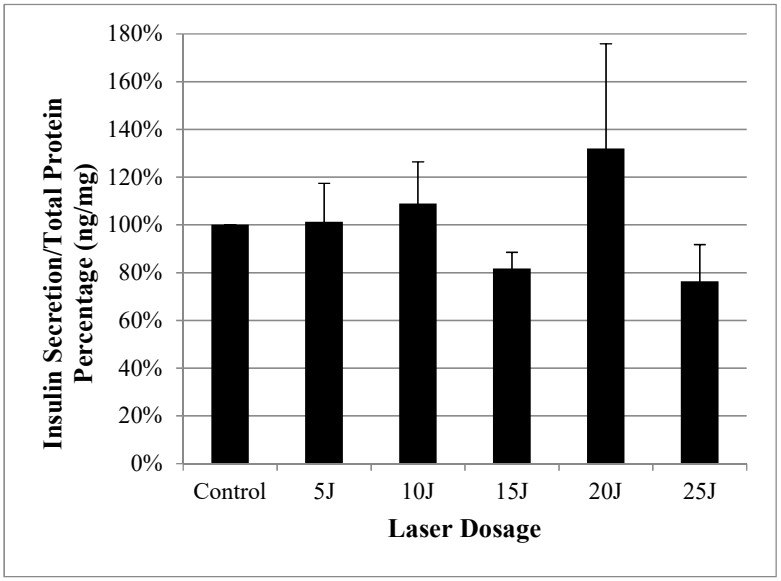
The combined data from Study 1 and Study 2 was utilized again and blocked by gender (n = 10, male; n = 6, female). Significant differences were found in the male islets in the low glucose media. Insulin secretion was increased in the male islets receiving the 5J laser dosage compared to the male control (P = 0.037), compared to the female islets receiving the 5J laser dosage (P = 0.031), and the male islets compared to the female islets exposed to the 10J laser dosage (P = 0.020) (Figure 6a). There were no significant differences seen in the high glucose media when comparing genders (Figure 6b).

Figure 1a. Percentage of Insulin Secretion/Total Protein (ng/mg) in Low Glucose Media (3.0 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 1 (n = 8)



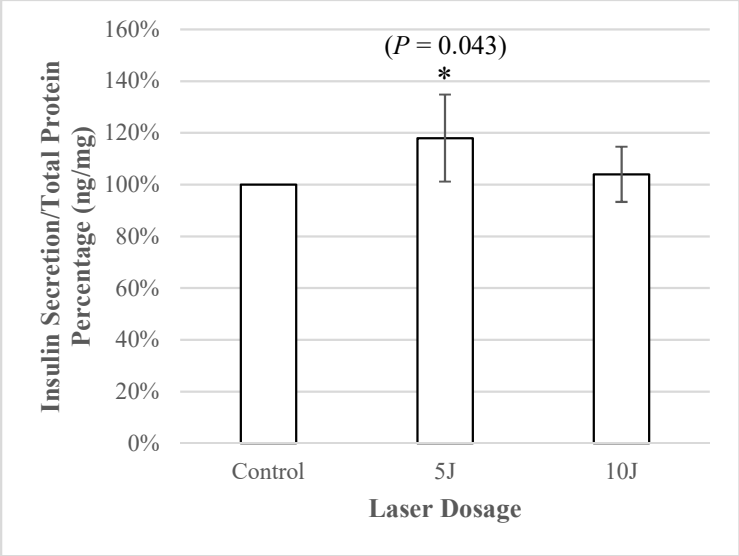
-All measurements were normalized to the control
-No significance differences were found ($P > 0.05$)

Figure 1b. Percentage of Insulin Secretion/Total Protein (ng/mg) in High Glucose Media (16.7 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 1 (n = 8)



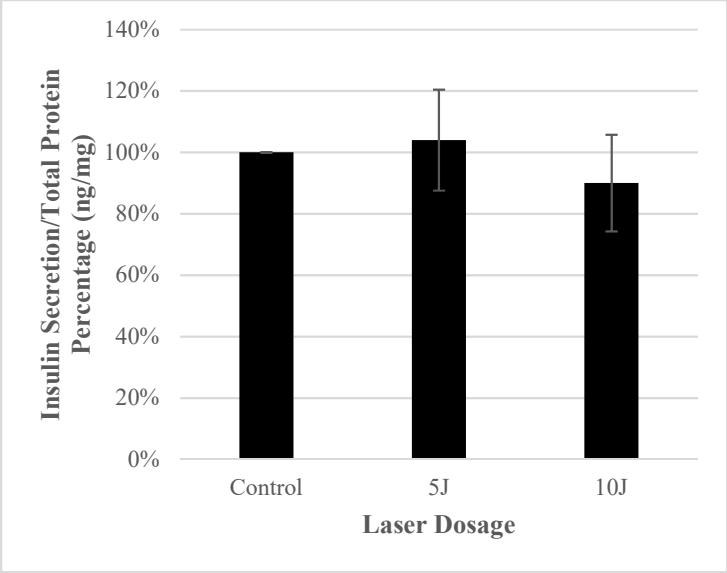
-All measurements were normalized to the control
-No significance differences found ($P > 0.05$)

Figure 2a. Percentages of Insulin Secretion/Total Protein (ng/mg) in Low Glucose Media (3.0 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 2 (n = 8)



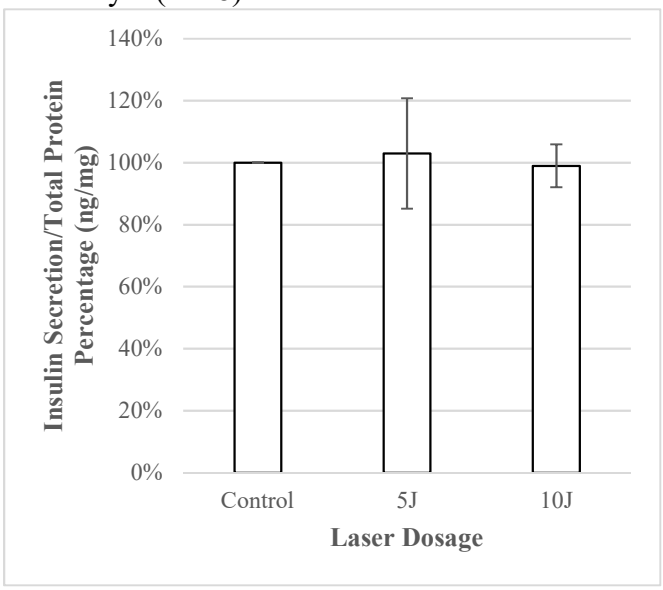
-All measurements were normalized to the control
-The bar with an asterisk (*) represents a significance difference
-Bars without an asterisk (*) represents no significance differences ($P > 0.05$)

Figure 2b. Percentages of Insulin Secretion/Total Protein (ng/mg) in High Glucose Media (16.7 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 2 (n = 8)



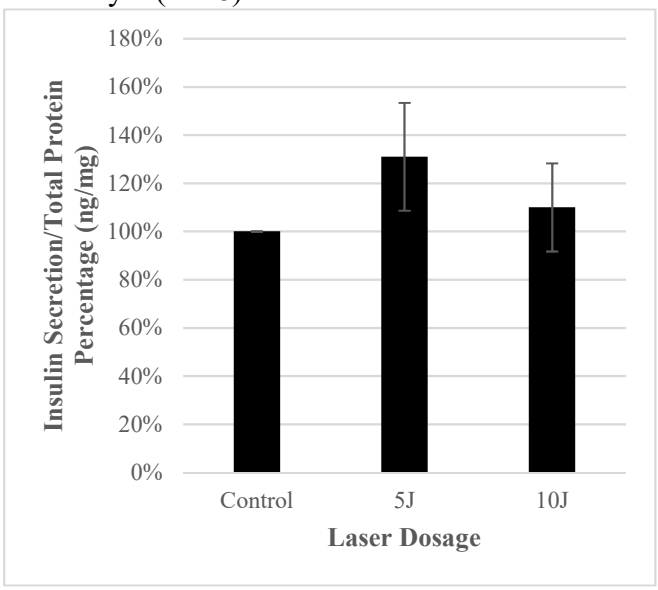
-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

Figure 3a. Percentages of Insulin Secretion/Total Protein (ng/mg) in Low Glucose Media (3.0 mM) at a 20-Minute Incubation Period Based on Different Dosages of LLLT 0633 nm) for Study 2 (n = 8)



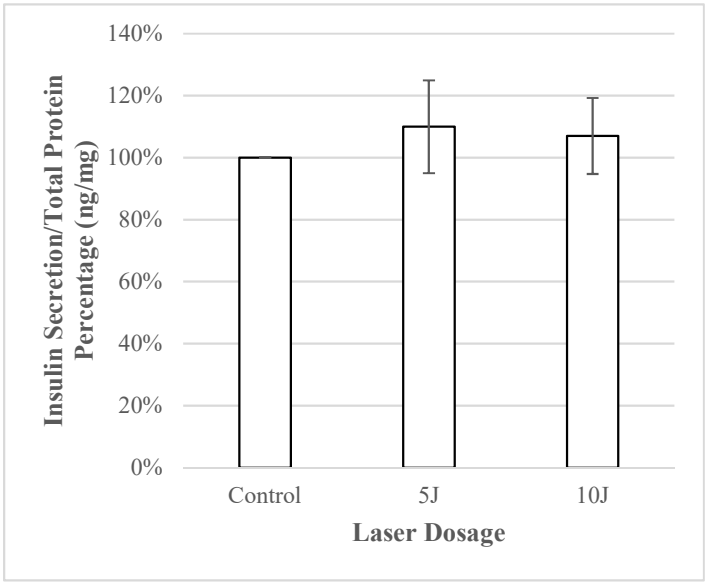
-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

Figure 3b. Percentages of Insulin Secretion/Total Protein (ng/mg) in High Glucose Media (16.7 mM) at a 20-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 2 (n = 8)



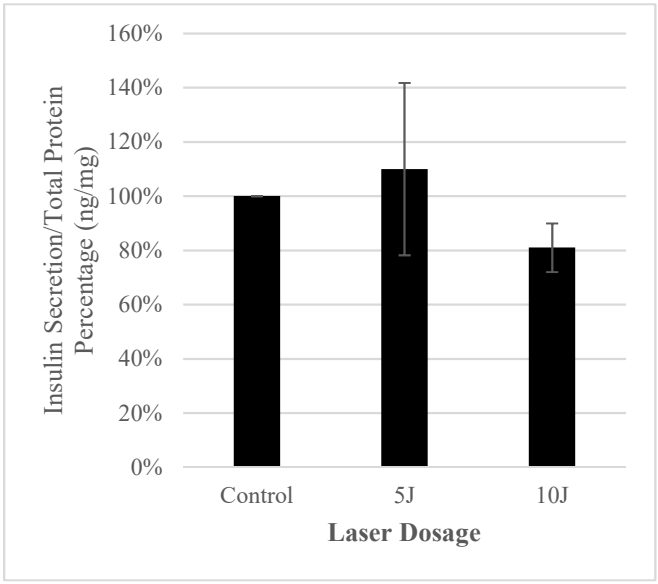
-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

Figure 4a. Percentages of Insulin Secretion/Total Protein (ng/mg) in Low Glucose Media (3.0 mM) at a 10-Minute Incubation Period Based on Different Dosages of LLLT (633nm) for Study 2 (n = 8)



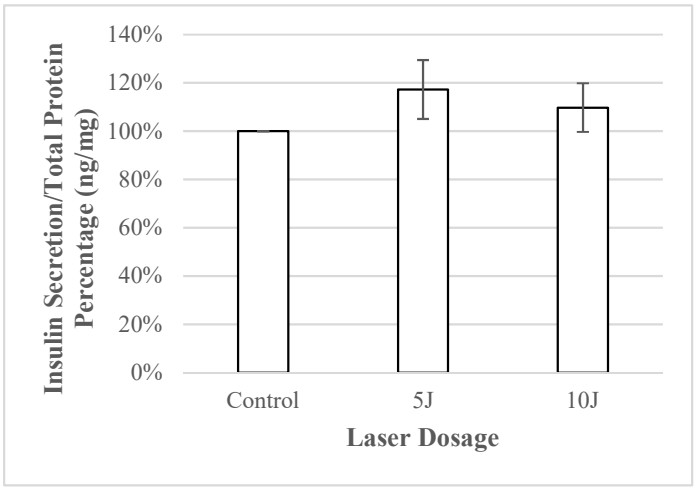
-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

Figure 4b. Percentages of Insulin Secretion/Total Protein (ng/mg) in High Glucose Media (16.7 mM) at a 10-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 2 (n = 8)



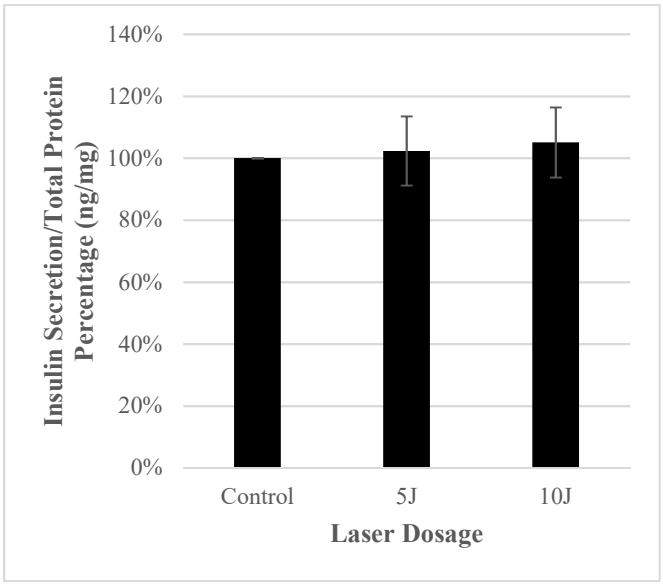
-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

Figure 5a. Percentages of Insulin Secretion/Total Protein (ng/mg) in Low Glucose Media (3.0 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 1 combined with Study 2 (n = 16)



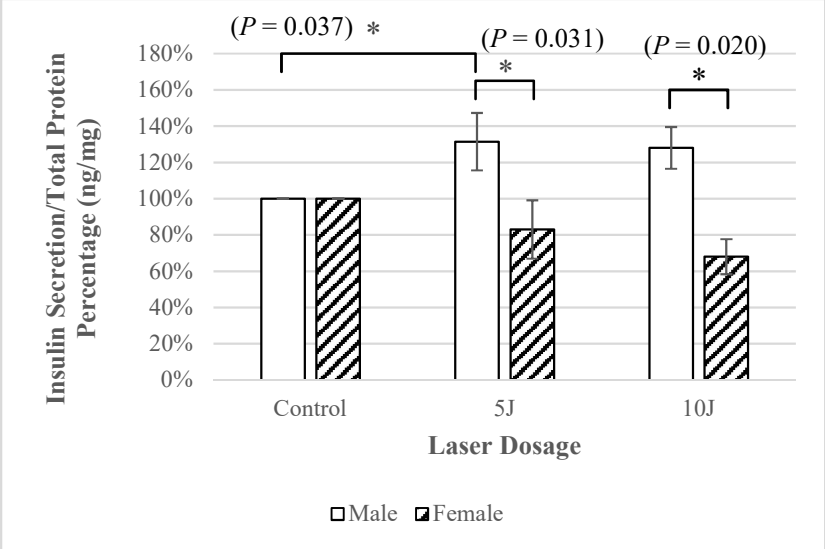
-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

Figure 5b. Percentages of Insulin Secretion/Total Protein (ng/mg) in High Glucose Media (16.7 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 1 combined with Study 2 (n = 16)



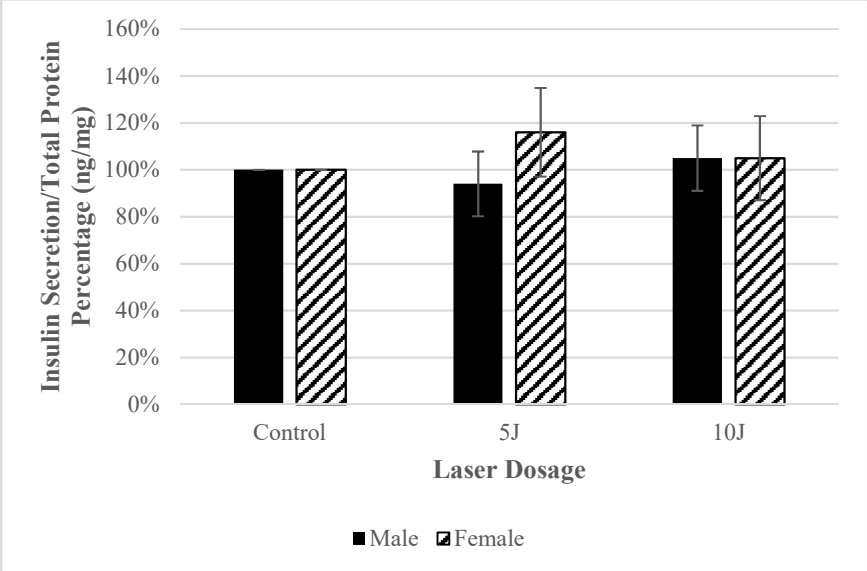
-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

Figure 6a. Percentages of Insulin Secretion/Total Protein (ng/mg) in Low Glucose Media (3.0 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 1 combined with Study 2 Blocked by Gender (n = 10, male; n = 6, female)



-All measurements were normalized to the control
-Bracketed bars with an asterisk (*) represent significance differences
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

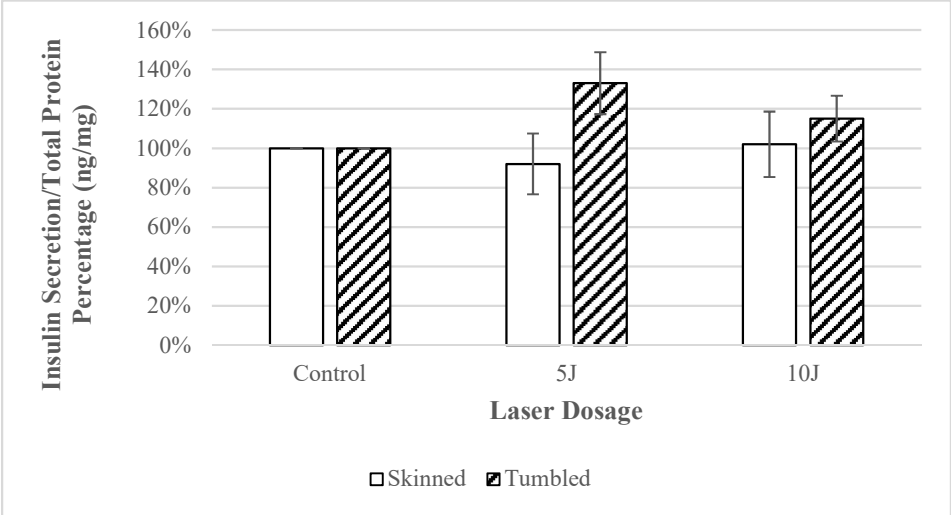
Figure 6b. Percentages of Insulin Secretion/Total Protein (ng/mg) in High Glucose Media (16.7 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT 9633 nm) for Study 1 combined with Study 2 Blocked by Gender (n = 10, male; n = 6, female)



-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

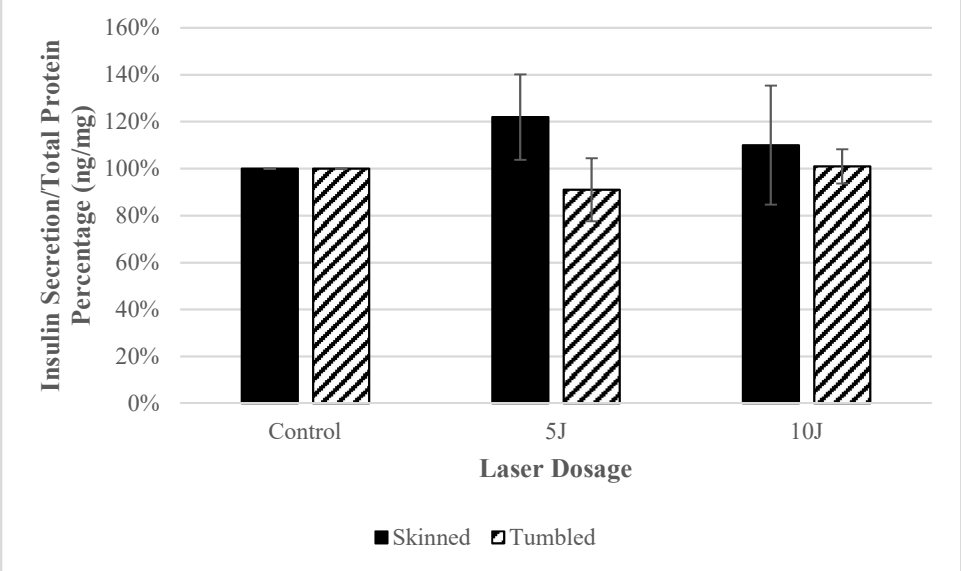
The combined data from Study 1 and Study 2 was blocked based on the different harvesting process (n = 10, tumbled; n = 6, skinned). This was dependent on whether the pig was placed in a tumbler to singe off the hair (tumbled) or if it was skinned which removes the hide during the harvesting process. This data comparison showed no significant differences found in the low glucose (Figure 7a) or in the high glucose (Figure 7b) when comparing harvesting processes.

Figure 7a. Percentages of Insulin Secretion/Total Protein (ng/mg) in Low Glucose Media (3.0 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 1 combined with Study 2 Blocked by Harvesting Process (n = 10, tumbled; n = 6, skinned)



-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

Figure 7b. Percentages of Insulin Secretion/Total Protein (ng/mg) in High Glucose Media (16.7 mM) at a 30-Minute Incubation Period Based on Different Dosages of LLLT (633 nm) for Study 1 combined with Study 2 Blocked by Harvesting Process (n = 10, tumbled; n = 6, skinned)



-All measurements were normalized to the control
-Bars without an asterisk (*) represent no significance differences ($P > 0.05$)

DISCUSSION

The objective of this study was to investigate whether laser therapy would increase insulin secretion on isolated porcine islets that were tested in low and high glucose media.

In study 1, no significant differences were found after a 30-minute incubation period under laser dosages between 5J and 25J. Although not statistically significant, the results from this study did show the islets receiving the 15J laser dosage released less insulin than the control, therefore it can be suggested that the dosages of 10J or less might be more promising and were focused on for study 2.

Past literature over other sensory cells also favored 10 and 20-minute incubation periods after laser treatment where peaks in secretion were documented (Oron et al., 2007, Karu et al., 1995). Therefore, the method for study 2 includes 30, 20 and 10-minute incubation periods to determine if incubation time could play a role in insulin secretion. The 5J laser dosage with a 30-minute incubation period in low glucose was the most optimal over the other dosages and incubation periods.

Therefore, the lack of significant differences found in study 1 could be due to the high variation in incubation time observed because of the initial design. Study 1 had a larger incubation interval between the first and last samples after the laser therapy because there were more samples within the microplate. Whereas, Study 2 had fewer samples within each microplate, resulting in less variance in the culture times between the first and last samples.

When combining study 1 and study 2 (n = 16 at dosages of 0J, 5J and 10J for a 30-minute incubation), no significance differences were found. Again, it is inferred that the high

variation observed within study 1 was too large to compensate for given the restricted sample size. However, a further analysis was conducted researching gender differences. The results from this study show a higher sensitivity in male islets versus female islets; as they had an increased response in insulin secretion to the laser therapy. There are limited articles on the differences of islets between genders. Only one study suggested that insulin content was similar when comparing male and female islets in mice (Strandell and Sandler, 1997). Therefore, this could be the first report showing a significant difference to stimulants such as LLLT in this study islet based on gender.

The viability of the islets was being questioned on the quality of the pancreas sample collected which varied based on the harvesting process. The tumbling process seemed to be slightly shorter, but not by much time. Simply comparing one process against the other showed no apparent differences found. Therefore, future research should not need to take into consideration the differences in the harvesting procedures.

All significant differences were observed in low glucose media rather than the high glucose media. In addition, high glucose did not seem to increase insulin significantly both with and without LLLT. This was in agreement with previous literature where isolated pig islets did not respond significantly to high glucose for insulin secretion (Cooper et al., 2016). However, LLLT does not seem to improve this characteristic either.

Limitations

Due to limited availability of porcine subjects and the sampling environment, there was variation within the pig selection process including harvesting the pancreas and the

purification of the islets. In order to retrieve enough yield of isolated islets for each trial, during the isolation process, there were non-islet cell tissue debris within the samples. There was also a time gap limitation in the laser therapy procedure; the first well had a longer incubation time versus the last well.

CONCLUSION

In conclusion, laser therapy significantly increased insulin secretion on pig islets in low glucose media at a dosage of 5J. Laser therapy also increased insulin secretion in male pig islets in low glucose at 5J compared to its control and at 5J and 10J compared to the female islets. Future research should further investigate modifications in the harvesting procedure that will allow for a shorter collection time of samples. There is also a need for an improved isolation process that result in more purified islets before transplantation is possible. The laser therapy should be focused on 10J or less; the 15J dosage and higher seemed to decrease the viability of the islets. Additional avenues of research include further investigation of the role gender plays on the viability and sensitivity of islets. A follow up study can focus on a perifusion system for the isolated islets which more closely mimics the environment found within the body.

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APPENDIX

	200 mM Glucose	Plain RPMI
Low Glucose (3.0 mM)	150 µL	4,850 µL
High Glucose (16.7 mM)	835 µL	4,165 µL

-200 mM Glucose = 1.8 g of Glucose in 1000 mL of Plain RPMI
-Low Glucose at 3.0 mM = 54 mg/dL = fasting glucose level
-High Glucose at 16.7 mM = 300 mg/dL = postprandial glucose level